v News & Features
InFocus
- Archives
> Research News
Industry News
Features
Pubmed

Events
Help

Genomics
Proteomics
Bioinformatics
Biotherapeutics
Bioengineering
Drug Discovery
Immunotech

DNA Throttle Controls Molecular Machine

9/5/2003 -- A DNA sequence that acts as a throttle to control the rate at which an enzyme moves along the DNA has been observed by researchers at UC Davis. By controlling the activity of the RecBCD helicase enzyme, the "Chi" sequence can affect how efficiently genes are repaired.

RecBCD unwinds the DNA double helix so that the genetic code can be read, copied or repaired. This unwinding is an essential first step in most processes involving DNA.

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The research findings, which are published in the September 5 issue of the journal Cell, could explain how short DNA sequences such as Chi can interact with enzymes and affect how DNA is copied or repaired. They could also give insight into how to control the speed of tiny nanomachines built for various purposes.

The enzyme moves along DNA at a rate of up to 1000 base pairs a second. Using special apparatus to film single enzymes at work in real time, the UC Davis researchers found that when RecBCD reaches the eight-letter Chi sequence, it stops for up to 10 seconds and then carries on at half speed.

The researchers attached DNA molecules labeled with a fluorescent dye to polystyrene beads one-millionth of a millimeter in size. Under the microscope, the bead looks like a white sphere with a bright string of DNA attached.

The researchers were postdoctoral scholars Maria Spies, Piero Bianco, Mark Dillingham and Naofumi Handa with Stephen Kowalczykowski, professor of microbiology and director of the UC Davis Center for Genes and Development, and Ronald Baskin, professor of molecular and cell biology.

They let RecBCD attach to the free end of the DNA strand, and used laser beams as "optical tweezers" to move the beads into position under a microscope.

As RecBCD unwinds the DNA strands, the fluorescent dye is removed, so the bright string of DNA appears to shorten.

When the researchers put RecBCD onto DNA molecules carrying the Chi sequence, they found that RecBCD stops for up to 10 seconds when it reaches the beginning of the Chi sequence, then continues at a slower rate.

"It's a complete surprise," Kowalczykowski said. The results would have been impossible to find with a conventional bulk experiment averaging the activity of many enzymes and DNA molecules, he said.

RecBCD is a molecular machine made up of three proteins. Two of these are motor units that propel the enzyme along the DNA double helix. Kowalczykowski believes that the change in velocity is due to one of two motor subunits in RecBCD being switched off by the Chi sequence.

The Chi sequence is known to be associated with "hotspots" where genes are readily exchanged, or recombined, between chromosomes.

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back top